

Confirming *Agrobacterium* with Benedict's Reagent

- ❑ Prepare a streak of the culture being confirmed on Lactose Agar. It is not necessary to include selective agents in the medium but allow 2-3 days growth since young cultures tend to have weak responses.
- ❑ Flood all or part of the plate with Benedict's Reagent (1 to 5 ml per plate is adequate).
- ❑ Wait for up 1-2 hours or until a bright, yellow product is observed in the agar. The yellow color confirms the presence of *Agrobacterium* sp. since *E. coli* does not convert lactose to 3-ketolactose. Absence of the yellow color may mean that the culture in question is, in fact, something other than an *Agrobacterium*. (A few biovars of *Agrobacterium* are unable to carry out the conversion).

Reagents:

Benedict's Solution¹

- Solution I

Mix the following:

17.3g Na Citrate
10.0g Na ₂ CO ₃
60-70ml hot deionized water (~55 deg.)

Solution II

1.73g /10 ml H₂O CuSO₄ · 5H₂O

Cool **Solution I**, add 10 ml **Solution II**, bring up to 100ml with deionized water. Stable at room temperature.

Lactose Agar

(*This is NOT the same thing as L-Agar or LB-Agar*)

Mix the following:

1g yeast extract
10g lactose
15g Agar

- add 1 liter deionized water.
- autoclave for 20 min.
- let cool to 55°C and pour into Petri dishes

¹ Bernaerts M.J. and De Ley J. (1963) A biochemical test for crown gall bacteria. Nature 167:406-407.